

Rosa Margesin · Franz Schinner (Eds.)

Cold-Adapted Organisms

Ecology, Physiology, Enzymology and Molecular Biology

With 100 Figures and 26 Tables

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Preface

Cold ecosystems cover the major part on earth and are colonized by cold-adapted microorganisms, plants and animals. These organisms have adapted to their environment in such a way that metabolic processes, reproduction and survival strategies are optimal. They are responsible for nutrient turnover as well as for production and decomposition of biomass in terrestrial and aquatic cold ecosystems.

Despite their important role, cold-adapted organisms have received little attention so far both in basic and applied research. In this book, prominent authors present fundamental and new knowledge and concepts regarding cold-adapted microorganisms, plants and animals in the fields of ecology, physiology, enzymology and molecular biology.

Beside of the ecological importance of cold-adapted organisms, their large potential for biotechnological applications has been recognized only recently. The interested reader is referred to our book "Biotechnological Applications of Cold-Adapted Organisms" (Springer-Verlag).

We are most grateful to the authors for their excellent contributions. This book has been produced within the Concerted Action "Eurocold" under the coordination of Professor N.J. Russell. We are also indebted to the publishers, especially to Dr. D. Czeschlik, for their cooperation, and to B. Marschall for skilful preparation of the text layout.

Innsbruck, January 1999

R. Margesin, F. Schinner

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Ecology and Physiology

Lake ice microbial communities in alpine and antarctic lakes

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1

Introduction

The observation that metabolic processes are reduced or completely inhibited at freezing temperatures has influenced microbial ecologists and limnologists, who have commonly concentrated on warm seasons and temperate habitats. High altitude and high latitude sites are not easily accessible and conducting research at low temperatures is not a trivial matter. However, methods for *in-situ* studies of microbial processes such as bacterial growth and production have been improved to a degree that allows the study of very oligotrophic systems at low temperatures. Furthermore, the construction of observatories and field stations has increased accessibility to high alpine and antarctic ecosystems, which has largely extended our knowledge of microbial life in cold environments.

Recent interest in cold ecosystems such as snow and ice has been stimulated by the recognition that global change will strongly influence these systems, and by the notion that ice dynamics in the oceans and inland waters can largely influence the emission of greenhouse gases.¹ The study of lake ice microbial communities, although their contribution to climate change is likely to be marginal, may foster the understanding of microbial communities in extreme environments, their functional role in the cycling of nutrients and organic matter, and their sensitivity to climate variations. The ice cover plays a major role in determining the environmental properties of lakes. It reduces heat exchange and prevents wind-generated mixing of the water column; this means that transport processes in the water column will eventually depend only on diffusion, thus ice influences not only the transfer of energy and matter between the atmosphere and the water, but within the lake

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itself.² Light transmission is severely limited by ice and especially by snow layers (by up to 99.9%) and atmospheric gases are trapped in ice-covered lakes which can result in severe oxygen deficiency³ and strong oversaturation of CO₂,⁴ N₂ and O₂.⁵ Nutrient input from the atmosphere is also inhibited by the ice cover and sedimentation fluxes can become very low. Thus, the formation of an ice cover triggers a switch in a lake's physicochemical dynamics and may be the major factor limiting productivity in the pelagic zone of ecosystems with long-lived or perennial ice covers.

In addition to their role as physical barriers and limiting factors for water column processes, ice covers on alpine and antarctic lakes contain active microbial communities which will be at the center of interest in this chapter.

2 Formation and characteristics of lake ice

2.1 Seasonal ice covers on high mountain lakes

Ice sheets on high mountain lakes in the Alps and the Pyrenees, lasting for half a year or longer, are generally characterized by slush layers located between solid ice created through dynamic flood-freeze cycles. The seasonal ice cover starts in general in a cold autumn night with the formation of black ice, which is optically clear and hard with nearly no inclusion of air bubbles. Snowfall on the black ice pushes it downward and lake water infiltrates the base of the snow cover – which is an excellent thermic isolator – through cracks. Freezing of the infiltrated water can create a solid layer consisting of snow crystals and frozen lake water (called white ice) on the surface of the black ice. Incomplete freezing of the infiltrated snow and subsequent flooding and freezing creates a sandwich-like structure consisting of alternating layers of snow, white ice, and slush on top of black ice (Fig. 1). Slush may be best compared to a sandy sediment but with grains consisting of ice crystals. The dynamic changes in the physical structure of these seasonal ice covers are driven by snow deposition, melting, freezing and flooding⁶ which is similar to those processes creating productive microbial habitats on the surface of sea ice in the Southern Ocean.^{7,8} The seasonal ice cover becomes thinner and structurally weaker during spring. This and the formation and widening of cracks enhances gas exchange with the atmosphere and increases sedimentation and light transmission. The increase in transmissivity can enhance photosynthesis of phytoplankton and, consequently, bacterial activity in the water column. The influence of the ice cover on pelagic life may, however, not cease with the complete melting, because the microbial communities developing in the ice column may provide an inoculum to the water column and thus influence composition and development of microbial communities in the open lake as suggested by Andreatta⁹ for lakes which are not very deep.

It is remarkable that the physical and chemical composition of the ice cover, though resembling a rigid structure, is constantly changing. Associated with physical transformations, the different layers undergo chemical changes that can lead to

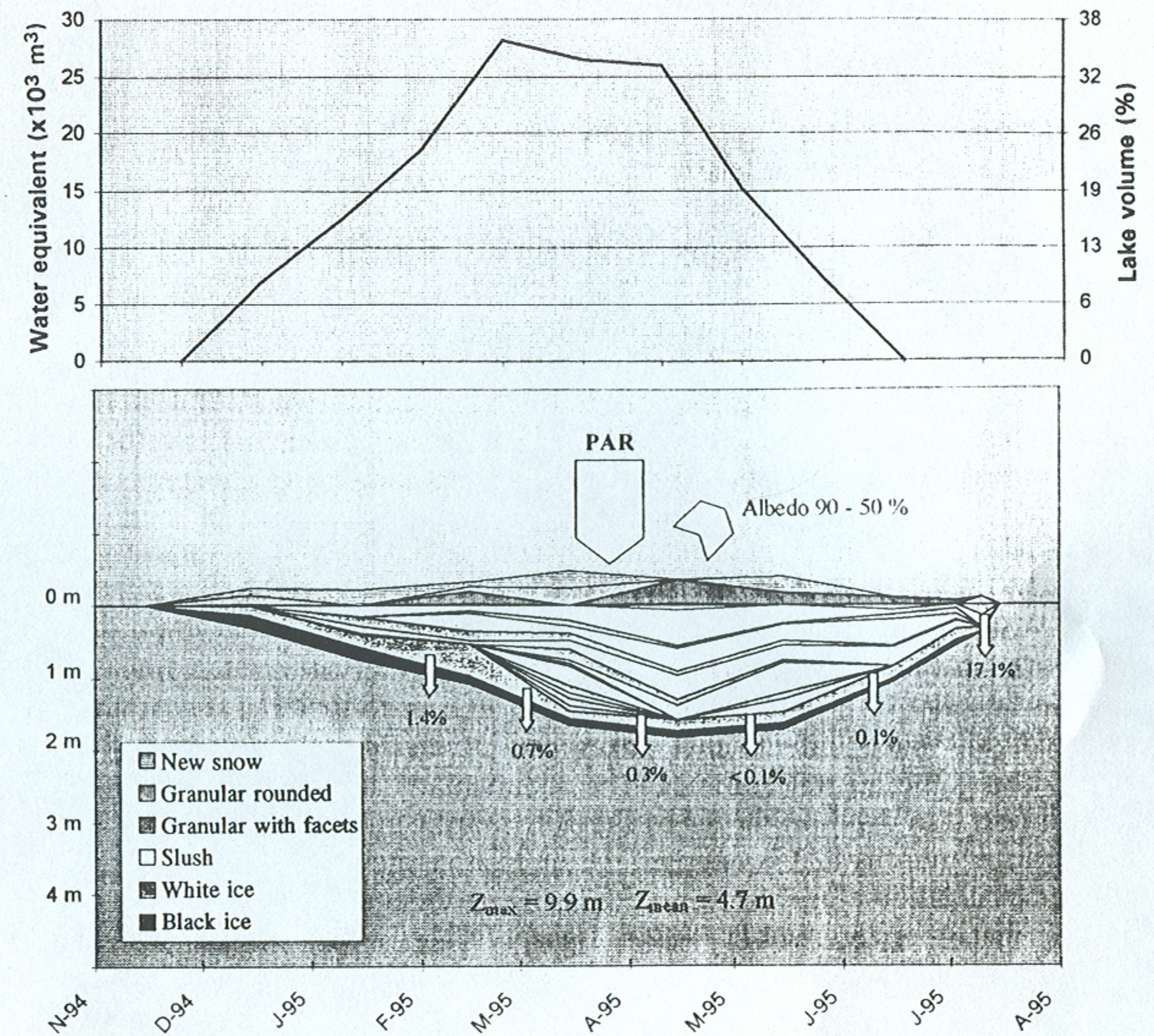


Fig. 1. Winter cover of a high mountain lake in the Alps (Gössenköllesee, Austria) (Upper panel) Water equivalent compared to the percentage of total lake volume (season 1994/95); (lower panel) Schematic representation of winter cover from ice formation until ablation with included slush layers. Arrows on top of the cover depict incoming photosynthetically active radiation (PAR) and outgoing albedo. Downward arrows show percentage of radiation after passing snow and ice layers; Z_{max} maximum depth; Z_{mean} mean depth

either concentration or dilution of dissolved and particulate matter by partial melting, washout or entrainment of material from the atmosphere, the catchment and the lake. As a result, slush layers enriched in nutrients (phosphorus, ammonium and nitrate) are common.^{6,10} At the end of winter, when light attenuation by snow decreases with thawing, light levels in the upper slush layers can be sufficient for net photosynthesis. In addition, seepage water from melting snowfields in the drainage area and atmospheric deposition can deliver organic matter and nutrients to the cover. The atmospheric input of pollen, conidia, leaves and stem debris, and of amorphous organic matter can become very high in springtime, when the lakes are still frozen but low altitude sites are free of snow. All these facts indicate that the ice and snow cover of lakes could support a microbial community, although the low

temperatures -0°C throughout the winter – may prevent them from reaching measurable activities.

2.2 Permanent ice covers on antarctic lakes

Aeolian deposition of sediments on the surface of the permanent ice covers is responsible for sorting and transporting lithogenic matter (primarily sand- and clay-sized particles) from the surrounding desert environments (soils, ephemeral streams, glaciers, etc.) onto the ice covers¹¹ that range in thickness from 3 to 20 m. These ice covers are cold (average temperature -10°C) and impermeable to materials for about 9 months out of the year. During the remaining 3–4 months of the annual cycle, the ice covers become relatively warm and isothermal at 0°C . Liquid water (a highly limiting factor for microbial proliferation in the polar desert) is generated near sedimentary aggregates in the ice due to the trapping of radiant energy by the relatively dark lithogenic and pigmented biogenic matter during this time.^{8,12} Small-scale dynamics of the sediment aggregation and localized melting processes have not been investigated in detail, yet observations and preliminary analysis at scales of meters suggest that the 3 to 5-m-thick ice covers attain substantial liquid water contents⁸ that makes them permeable to lithogenic matter and gases during the austral summer. The sedimentary aggregates are thought to remain near 2 m depth in the ice by the downward melting and migration during the summer months that is opposed by the relative movement of the ice upward (caused by ablation at the surface and freezing on the bottom).¹³ The combination of these dynamic geophysical processes is responsible for the existence of seasonally transient liquid-water habitats that are characterized by the continual presence of ice surfaces, lithogenic sediments, low temperatures (0°C), annual freeze-thaw cycles, and extreme seasonal variability in solar radiation.

2.3 Comparison of lake ice structures

A comparison of mountain lake and antarctic lake ice structures is shown on Figure 2, which highlights similarities and differences in the habitats: the most obvious distinction is the layered structure of the ice covers in mountain lakes of the Alps and the Pyrenees, with a duration from 6 to 9 months, and the patchy distribution of sediments and water pockets in the Mc Murdo Dry Valley lakes within its permanent ice cover. These distinctions are primarily determined by the lower annual temperatures in the antarctic setting as well as the low amount of precipitation (below 100 mm) in the polar desert.

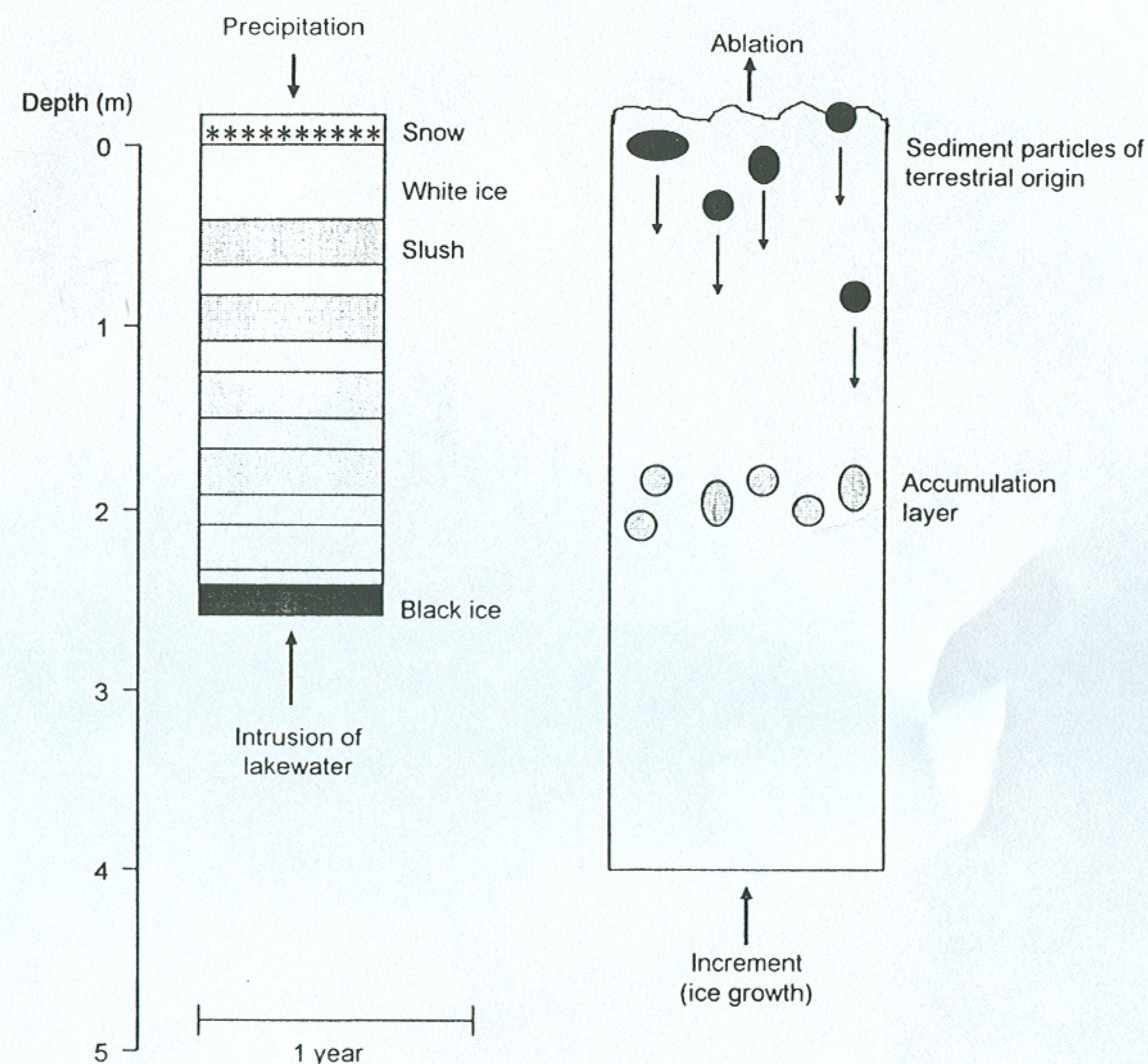


Fig. 2. Winter cover of high mountain lakes in the Alps and the Pyrenees (*left*) and permanent ice cover of antarctic lakes in the Mc Murdo Dry Valley region (*right*)

3 Composition, origin, morphology and activity of lake ice microbial communities

3.1 Alpine lake ice microbial communities

The infiltration of lake water into the ice cover transports organisms, inorganic nutrients and other lake water constituents into the snow and ice, where they often encounter more light than below the ice cover. Microorganisms found in slush layers, however, originate also from atmospheric and terrestrial sources. During the study of lake ice microbial communities (LIMCOs) we realized that fresh snow and supercooled cloud droplets collected at high altitudes contain active bacteria

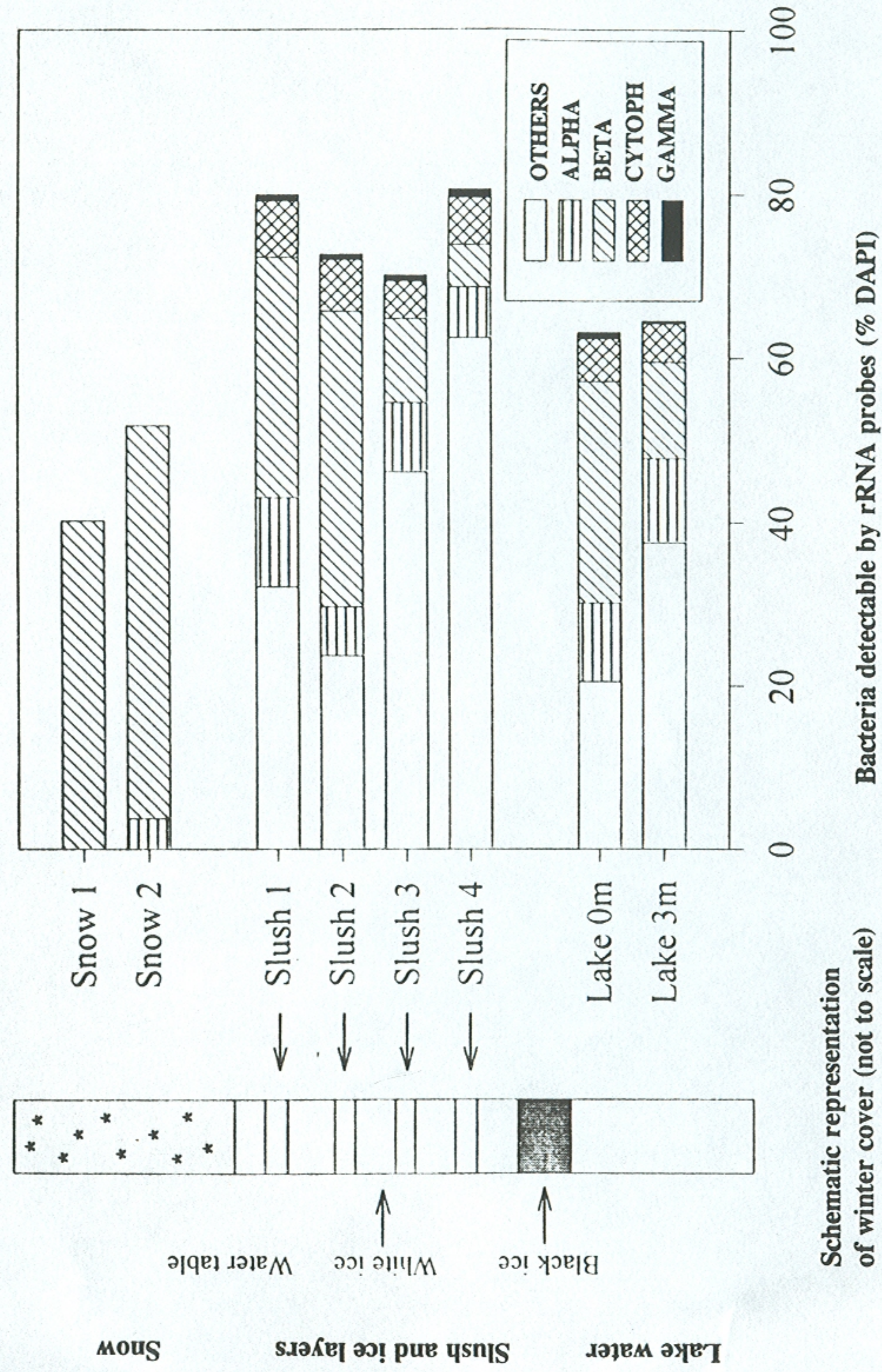


Fig. 3. Bacterial assemblages in snow and slush layers of the winter cover and two pelagic layers of a high mountain lake. Determination after *in-situ* hybridization with group-specific oligonucleotide probes (redrawn after ref. 14)

ALPHA complementary to the 16S rRNA (positions 19–35) of α -subclass Proteobacteria; BETA β -subclass Proteobacteria; BETA β -subclass Proteobacteria (23S rRNA, positions 1027–1043); CYTOPH *Cytophaga-Flavobacterium* group (16S rRNA, positions 319–336); GAMMA γ -subclass Proteobacteria (23s rRNA, positions 1027–1043); DAPI staining with 4,6-diamidino-2-phenylindole

(unpubl. data), and Alfreider et al.¹⁴ showed that the taxonomic composition of snow bacteria differed considerably from those found in slush layers or lake water (Fig. 3). These findings have led us to the assumption that microbial processes might not be restricted to lake ice habitats but that bacteria are metabolically active before the deposition of rain and snow. This hypothesis is supported by the findings of Puxbaum (unpubl. data), who showed that bacterial metabolites can be found in cloud droplets which may persist for several hours or days at temperatures below 0°C in a supercooled state. Thus, airborne bacteria may be the first organisms colonizing snow fields and may have an influence on the ecology of snow-covered remote environments such as high-altitude lakes and soils.

Bacterial communities are also morphologically diverse in slush layers and lake water. Water column bacteria are generally small free-living cells, mostly short rods and cocci (about 0.05–0.1 μm^3). In slush layers, however, together with these small forms, long thin filaments appear, sometimes longer than 100 μm , which are the dominant organisms regarding bacterial biomass. Long branched filaments and stalked bacteria are not uncommon, and in a few cases *Ancalomicrobium*-like morphologies are found. *In-situ* hybridization with fluorescent labeled rRNA-targeted oligonucleotide probes was carried out by using probes specific for all members of the domain bacteria, for the α -, β - and γ -subclasses of the class Proteobacteria, and for the *Cytophaga-Flavobacterium* group to obtain information concerning bacterial community structure. Results showed a distinct bacterial community composition in the different snow, slush and pelagic layers (Fig. 3).

Ciliates typical in high alpine lake water include *Askenasia chlorelligera*, *Urotricha* sp. and *Balanion planctonicum*, whereas slush layers are characterized by nonplanktonic genera such as *Urosoma*, *Lacrymaria* and *Dileptus* and some unidentified species. The only autotrophic species exclusive to the cover are those found in surface pools (*Chlamydomonas nivalis*, an unidentified *Chlamydomonas* sp., and an unidentified *Chromulina* sp.). Nonflagellates seldom appear in the slush layers. Other autotrophs found in slush are *Rhodomonas minuta*, *Gymnodinium* sp. and *Chromulina* sp. which are the dominant algae (Büsing, unpubl. data), but appear also in the pelagic zone of the lake. Felip et al.¹⁵ have shown that the species composition (including phytoplankton, heterotrophic flagellates, and ciliates) of slush layers, surface pools and lake water in Estany Redó in the Pyrenees is clearly distinct in each case, and this difference persists in different years.

Bacterial production and respiration in slush layers generally increase with growth and development of the winter cover and range from values characteristic for ultraoligotrophic to eutrophic lakes. Organisms living at low temperatures have higher nutrient requirements for sustenance of metabolic activities. Pomeroy et al.¹⁶ and Wiebe et al.¹⁷ found that marine bacteria living at 0°C can be substrate-limited as their growth was stimulated by the addition of nutrients and organic compounds. Felip et al.¹⁸ pointed out that high growth rates can be observed at low temperatures if the amount of substrate is elevated. Therefore, high nutrient concentrations may be necessary in cold-water environments in order to compensate for the low temperature constraints on metabolic processes and growth. Experiments to determine temperature optima of water column and ice bacteria indicate that bacteria living in slush layers are adapted to temperatures from 0 to 5°C; pro-

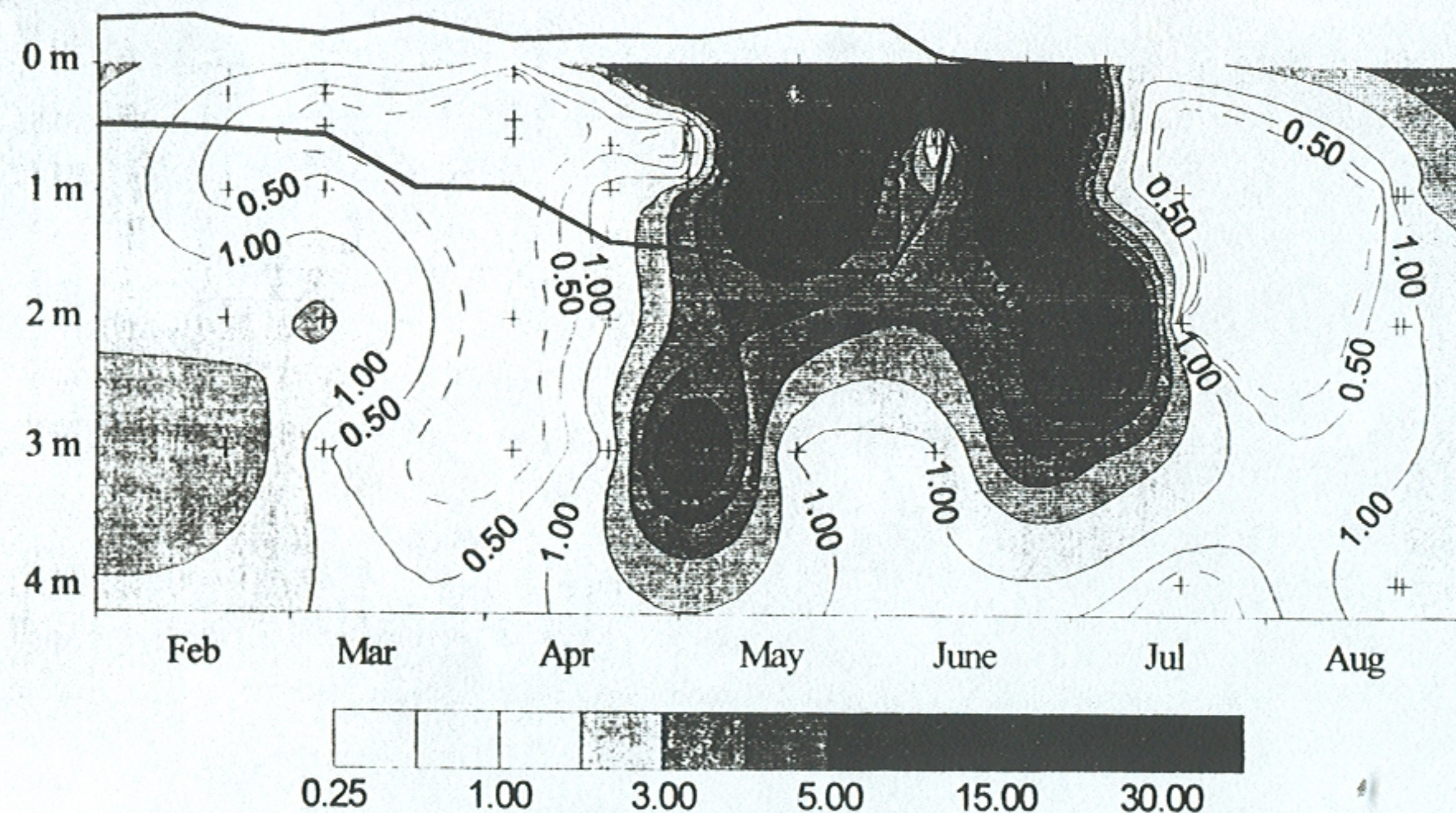


Fig. 4. Ratio of net primary production to bacterial production in slush layers of the winter cover (bold line) down to 4 m of the free water column in Gössenköllesee (Austria)

duction was decreased dramatically with rising temperatures. Lake water bacteria show an opposite trend, their preferred temperature range is near 15°C. Experiments indicate there is a taxonomic and physiological difference between the bacterial assemblages in the winter ice cover and those in the underlying lakewater (Fig. 3).

High nutrient concentrations in slush layers could lead to a stimulation of bacterial growth. Slush layers are inhabited by algae which support bacteria through extracellular release of carbon during high photosynthetic activity. From the formation of the ice cover to its decay we observed a shift from a system dominated by heterotrophs to an assemblage dominated by autotrophic organisms. This progression has consequences for the water column after ice break-up (Fig. 4).

Another effect favoring high microbial biomass inside slush can be the low density of grazers which may exert little grazing pressure. During such periods, microbial interactions in the ice cover are dominated mainly by bacteria and algae, thus being more similar to the antarctic lake ice than to the lake water itself. In a Pyrenean lake, however, Filip (unpubl. data) found higher numbers of heterotrophic nanoflagellates and ciliates, and also mixotrophic algae which may efficiently graze on bacteria. One possible reason for the low numbers of protistan grazers found in a lake in the Alps might be the reduced mobility due to the sediment-like structure of slush layers which can prevent effective grazing, while it is not known if the low temperature is a limiting factor for protistan activity. Sandwich-like structures and ice grains may also limit the access to metazoa, which were only occasionally observed in the winter cover of lakes in the Alps and the Pyrenees. The virtual absence of metazoa seems to be a common feature in antarctic lake ice but a remarkable contrast to the importance and densities of crustaceans in sea ice¹⁹ even at very low temperatures. As mentioned before, ciliate species typical of sediments

occur in the slush, which has led us to the assumption that the slush habitat resembles conditions in benthic strata or sandy sediments rather than in the pelagic zone.

3.2

Antarctic lake ice microbial communities

The community composition in the permanent ice covers on antarctic lakes also differs from the microbial communities found in the underlying water columns. The plankton within the liquid water column of permanently ice-covered antarctic lakes are typically composed of autotrophic eukaryotes (predominantly Chrysophytes, Cryptophytes, and Chlorophytes),^{20,21} but also of cyanobacteria, bacteria^{20,22} as well as heterotrophic flagellates and ciliates.²³ The phytoplankton are dominated by eukaryotic species that develop highly stratified distributions believed to be a result of preferential growth and survival of the different species along strong physicochemical gradients.²⁴ In contrast to the water column, lake-ice communities contain a mixture of heterotrophic bacteria, fungi, cyanobacteria, autotrophic flagellates and diatoms. Despite these ecologically functional members, the antarctic ice covers, the autotrophic biomass and activities are largely dominated by the filamentous cyanobacteria.^{25–27} The filamentous cyanobacteria assemblages in the permanent ice covers can potentially originate from pelagic, benthic, atmospheric and terrestrial sources. However, they more closely resemble those genera (e.g. *Phormidium* and *Nostoc*) found in ephemeral streams and ponds, wetted soils in the surrounding desert, and on the lake's benthos. Cyanobacterial mats in these environments have been shown to be extremely resistant to desiccation and freezing^{28,29} and are presumed to be capable of colonizing and growing within the extreme *in-situ* conditions of the permanent ice covers (e.g. long periods of freezing, freeze-thaw cycles, cold temperatures). Aeolian transport and deposition of sediment-associated cyanobacterial assemblages is likely to be the mode of introducing these assemblages into the ice covers.

Activities of microbes from several permanent ice covers has been examined at the time of ice melting. Active metabolic incorporation of radiolabeled substrates (e.g. ³H-thymidine and ¹⁴C-bicarbonate) was detectable within minutes to hours of encountering liquid water under laboratory conditions (i.e. irradiance >100 μmol photons m⁻² s⁻¹ and temperatures of 0–2°C) and enhanced bacterial and photoautotrophic biomass and activities were always found in ice meltwater containing ice-bound sediments.^{30–32} Ice covers lacking substantial sediments (Lakes Vanda and Morning) had 10- to 1000-fold lower heterotrophic and photoautotrophic activities (Table 1). Note that the rates reported in Table 1 depict the depth-integrated values. However, the activity is presumed to have been restricted to the aggregates that were only 1–10 cm in diameter and were scattered throughout the ice covers. Therefore rates on a volumetric basis should be scaled upwards severalfold; this illustrates that microbial processes in these environments are likely to be highly concentrated.

The low ratio of bacterial production to primary production in the ice covers with sedimentary aggregates (Table 1) suggests that microbial assemblages in these ice covers possess the immediate capability for net community growth based on

Table 1. Standing stocks of antarctic lake-ice constituents (sediments, chlorophyll *a* and bacterial cells) and rates of primary production and secondary bacterial production in ice core meltwater from permanent ice covers on lakes in the McMurdo Dry Valleys, Antarctica

Lake	Sediment wt (kg m ⁻²)	Chl <i>a</i> (mg m ⁻²)	Bacteria ^a (x10 ¹² m ⁻²)	BP (µg C m ⁻² d ⁻¹)	PPR (µg C m ⁻² d ⁻¹)	BP:PPR
East lobe Bonney	8.3	3.51	2.28	51.4	193	0.27
West lobe Bonney	1.4	1.33	1.11	27.8	27	1.02
Fryxell	8.9	7.42	6.00	504.1	1954	0.26
Hoare	8.8	5.25	2.12	235.3	2737	0.09
Joyce	7.2	5.08	0.76	56.5	580	0.10
Miers	11.4	22.9	7.43	989.2	33927	0.03
Morning	0.9	ND	3.66	43.3	ND	---
Vanda	0	1.20	0.62	5.9	2.9	2.07
Vida	22.2	5.05	10.0	138.9	1165	0.12

^a Determined via epifluorescence.

Wt weight; Chl *a* chlorophyll *a* (determined fluorometrically)²⁸; BP bacterial production (estimated from the incorporation of ³H-thymidine into trichloroacetic-acid-insoluble material)²²; PPR primary production (determined by the uptake of ¹⁴C into particulate organic carbon)²⁶; ND below detection

autotrophic production of new biomass when solar radiation and liquid water becomes available within the ice.

Temperature records have shown that liquid water is not likely to exist in the ice covers until late November or early December.⁸ Irradiances in the ice at this time are near their seasonal maximum and are well above the irradiance levels that saturate photosynthesis by the ice cyanobacteria. Therefore, community metabolism is likely to be supported by *in-situ* photoautotrophic production during the initial stages of the summer growth season. The seasonal progression of production and population dynamics of these communities throughout the summer growth season are unknown at this point in time.

4

Comparison between alpine and antarctic lake ice

A comparison between alpine and antarctic lake is given in Table 2 which summarizes the most important factors and processes leading to the formation of lake ice structures and microbial communities. Although antarctic lake ice is a permanent structure – in contrast to alpine lakes where the ice normally waxes and wanes in the course of 1 year – both systems are active sites of life for a period of only 5–7 months year⁻¹. Large differences exist also in the composition and the origin of organisms.

5

Conclusions and open questions

Is lake ice an ecosystem, an ecotone, or a habitat? In both alpine and antarctic lakes, it contains organisms which originate from the lake water, the atmosphere and the terrestrial catchment. These organisms form new assemblages in a physically complex and structured environment. The winter cover of mountain lakes can best be compared to a seasonally recurring habitat or a periodic ecotone. The occurrence of ciliates which are normally found only in benthic or terrestrial environments supports the idea of an ecotone linking atmospheric, aquatic and terrestrial systems. The relatively strict separation into different layers and the clear distinction of lake ice communities from those in surface pools and the pelagic zone¹⁵ confirms the hypothesis that they are at least very distinct from plankton, although the first settlers in the ice cover immigrate from the pelagic zone. In the end, i.e. when the ice cover melts, they merge to form new pelagic biota. This does not normally happen in the ice cover on antarctic lakes, which can nonetheless be considered an intermittent habitat, though consisting of permanent ice, because liquid water and measurable life processes occur only during the austral summer. Both systems are microbial worlds. Whether antarctic lake ice is an ecotone or not is more debatable, but it allows bacterial life of mostly terrestrial origin to develop in a new environment, which would otherwise not happen because of the dry and cold conditions in the surrounding soils.

Table 2. Characteristics of alpine and antarctic lake ice

	Winter cover of alpine lakes	Antarctic lake ice
Location	High mountain lakes (Alps, Pyrenees, etc.)	Lakes in the Mc Murdo Dry Valley
Formation	Lake water penetrates through the ice cover; snow, rain and melting water trickles down; Annual precipitation: 1,000–2,000 mm	Freeze-out of lake water; Annual precipitation: <100 mm
Thickness	1.5–3 m	3–6 m
Structure	Mixture of lake water, meltwater and rain water with snow and ice crystals; Sandwich-like structure	Uniformly hard lake ice with soil and rock particles surrounded by liquid water during summer
Duration	6–9 months: November through July	Permanent, but liquid water only in summer
Liquid water	10–30%; consists of lake water, rain and meltwater	About 10%, but only in summer around particles
Diffusion constant	Reduced: interstitial water, high tortuosity	Changing: melt-freeze intervals, sand clusters in water pockets
Radiation	Strong light gradient, from very high to <0.1%; UV-B radiation ca. 50% higher than at sea level	Strong light gradient, +/- constant radiation in summer; Low UV-B except during ozone hole events
Origin of organisms	Lake water, airborne, soils, littoral sediment	In-blown microorganisms from surrounding soils and long-range transport
Animals	No	No
Main feature	Intermittent (about 9 months); constantly at 0°C; Sandwich structure; "Sediment" of snow-ice particles; High bacterial production compared to lake water but reduced predation pressure; Microbial world: LIMCO (pro- and eukaryotes) originating from different sources	Permanent, but most time frozen; liquid water and microbial activity only in summer; Activity restricted to water pockets (patchy distribution); Microbial world consisting predominantly of cyanobacteria and heterotrophic bacteria; Eukaryotes are rare; Predation is almost absent

What our results clearly show is that temperatures at the freezing point do not inhibit the proliferation of microbial communities. Rather, active and diverse microbial communities develop in ice covers of distinct geographical regions of the world. The role of these communities in their respective ecosystems is still under study. In alpine lakes where the volume of the ice cover comprises a large portion of the total lake volume, the ice microbes may produce a substantial fraction of the

ecosystem's biomass during extended periods of the year. In permanently ice-covered lakes in Antarctica, the contribution of the ice communities to the overall productivity of the lake ecosystem is relatively small.

The increase in UV-B radiation in the Antarctic as well as in the northern hemisphere is a new challenge for microorganisms in highly exposed environments. While pigmented organisms seem to be rather insensitive to UV radiation³⁴ it is still an open question how bacteria will react upon an increase of UV radiation. Therefore, while still at the beginning of the exploration of microbial communities in snow and ice,^{10,15,24} we face a rapid change in those factors which are crucial for the formation, duration and physicochemical conditions of ice habitats, e.g. global warming, atmospheric deposition of acids and dust, and UV radiation.³⁵ We believe that it will be rewarding to investigate these habitats more intensively, for both a better understanding of ecological interactions under extreme environmental conditions and also for the investigation of microbial diversity and potential utilization of interesting traits.

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